Biting behaviour of *Anopheles funestus* populations in Mutare and Mutasa districts, Manicaland province, Zimbabwe: Implications for the malaria control programme

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**ABSTRACT**

**Background & objectives:** Biting behaviour of *Anopheles funestus* in Mutare and Mutasa districts, Zimbabwe, is little understood. An investigation was conducted to primarily compare indoor and outdoor biting behaviour of the mosquito, as well as blood meal sources and sporozoite rates.

**Methods:** Monthly adult anopheline sampling was conducted from October 2013 to September 2014 using Centers for Disease Control light-traps, pyrethrum spray catch and artificial pit shelter methods. Mosquitoes sampled by light-traps were divided into two cohorts. In one cohort, traps were left overnight and mosquitoes were collected the following morning, while in the other set, mosquitoes were collected hourly from 1800–0600 hrs. Collected females were identified using morphological characters and categorised according to their abdominal status. Polymerase chain reaction was used to identify *An. funestus* sibling species and blood meal sources. Infection rate was tested by enzyme-linked immunosorbertent assay.

**Results:** Morphological identification showed that indoor and outdoor catches comprised *Anopheles funestus* (98.3%) and *Anopheles gambiae* s.l. (1.7%). Of the 2268 mosquitoes collected, 66.2% were caught by light-traps, and 33.8% were caught resting indoors and outdoors. *Anopheles funestus* and *An. gambiae* s.l. were trapped more abundantly indoors (68%) than outdoors (32%). Both indoor and outdoor *An. funestus* densities were higher in wet (4.3) than dry season (1.8). In Burma Valley and Zindi areas, *An. funestus* demonstrated variable nocturnal indoor and outdoor flight activity rhythms, with two peaks during the night; between 2200–2300 hrs and 0200–0400 hrs. Human blood index in *An. funestus* was 0.64, with *Plasmodium falciparum* infection rate of 1.8%.

**Interpretation & conclusion:** The present work highlighted important information on the host-seeking behaviour, blood meal sources and infection rates in *An. funestus*. The information would be helpful in improving the vector control strategies.

**Key words** *Anopheles funestus*; biting behaviour; malaria; resting behaviour; Zimbabwe

**INTRODUCTION**

*Plasmodium falciparum, P. malariae* and *P. ovale* are the major human malaria parasites prevalent in Zimbabwe, of which *P. falciparum* constitutes 95% of the total morbidity and mortality¹. While *Anopheles arabiensis* is the principal vector of malaria parasites in Zimbabwe², *An. funestus* sensu stricto (hereafter referred to as *An. funestus*) is the primary vector in Mutare and Mutasa districts³. *Anopheles funestus* which prefers to breed in permanent or semi-permanent water bodies, generally exhibits patchy and discontinuous distribution pattern, is highly anthropophilic, and responsible for major malaria episodes in sub-Saharan Africa⁴. This is more likely due to increased human-vector contact as a result of human settlements being usually located near to permanent or semi-permanent water bodies.

In Zimbabwe, indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) are the major intervention measures to interrupt human-vector contact and malaria transmission. Mass distribution campaigns of LLINs have played an important role in reducing human-mosquito contact with consequent recent successes in malaria control⁵. LLINs protect against mosquito bites that mostly occur indoors at night when people are sleeping. The host-feeding activities of mosquitoes have important implications, since it is by this behaviour that the transmission of malaria and other vector-borne disease-causing organisms takes place. Studies on host preference, indoor and outdoor host-seeking behaviour as well
as biting times of mosquitoes are important in determining current mosquito habits, and form the basis for developing methods of personal protection against bites by vector mosquitoes.

Pyrethroid resistance in mosquito vector populations in most African countries is increasingly threatening the gains made by the use of pyrethroid-treated LLINs. In Temotu province, the Solomon Islands, An. farauti was reported to avoid insecticide exposure on the nets by shifting from feeding indoors (endophagic) to outdoors (exophagic)7. Evidences from recent studies in Africa suggest that malaria vector mosquitoes may avoid contact with insecticide imbedded in nets by biting predominantly outdoors or early evening and/or morning7-8. Russell et al9 documented evidence of increased proportions of outdoor feeding among malaria vector populations following scaled-up use of insecticide-treated nets in rural Tanzania. This modification in mosquito behaviour that helps to avoid lethal effects of insecticide may result from the selection of genetically-inherited traits in response to increased coverage of LLINs and/or IRS. Such inherited traits may render LLINs and/or IRS less effective in combating malaria transmission8.

While Anopheles mosquito feeding behaviour has been studied extensively in the Afro-tropical region; host preferences, biting rhythms and infection rates of the An. funestus populations remain poorly understood in Zimbabwe. These are important entomological indicators which guide vector control strategies.

In Gokwe and Binga districts, Zimbabwe, An. arabiensis has been reported to feed indiscriminately on humans and animals indoors and outdoors, illustrating behaviours where some mosquitoes feed on any available blood source rather than looking for the preferred host10. The work by Dandalo11 in 2007 on An. gambiae sensu lato (s.l.) in Gokwe South district in Zimbabwe showed the peak biting times to be from 2100 to 2200 hrs. Changes in mosquito biting behaviour observed in Gokwe and Binga districts, Zimbabwe10-11, and elsewhere in Africa7-9 might complicate studies on the determination of the feeding venues and times of vector mosquitoes as well as the ease with which LLINs can be implemented to combat malaria transmission. The behavioural characteristics of vector mosquitoes in malaria transmission may be different from region to region in Zimbabwe, and can well be understood only in the local context of available hosts, blood source preferences, behavioural resistance, and additional vector species.

Continuous monitoring and understanding behavioural responses of different vector mosquitoes to control interventions is crucial to the national malaria control programme (NMCP) as this facilitates the selection of the most effective control strategies. As Zimbabwe has joined the list of countries working towards eliminating malaria by 203012, the need to understand the biological implications of increased distribution of LLINs is of paramount importance. After the scaled-up implementation of LLINs project by the NMCP in Mutare and Mutasa districts, it is important to understand the behavioural responses of the major malaria vector, An. funestus, to this intervention measure. The aim of this study was to characterise the host-seeking behaviour of An. funestus by determining the human blood indices, host preferences, and sporozoite infection rate. The results presented here represent the first study on biting behaviour of the An. funestus populations in Zimbabwe.

**MATERIAL & METHODS**

**Study sites**

The field work was conducted in the villages of Burma Valley (19°11’S, 32°48’E; elevation 679 m) and Zindi wards (18°22’S, 32°56’E; elevation 766 m) in Mutare and Mutasa districts, respectively, of Manicaland province, Zimbabwe. The two areas are separated by a distance of about 200 km and extend to the Zimbabwe-Mozambique border. The sites have a combined population of about 13,880 people (Burma Valley 4,506 and Zindi 9374)3 whose major economic activities are agro-based. The people in the Zindi villages practice semi-commercial agriculture, with several plantations and estates that provide employment. A small number of people in Zindi are smallholder growers of coffee, tea, and banana plantations, while Burma Valley occupants mainly earn their livelihood by working in tobacco farms and to a small-scale, banana plantations.

The prevailing climatic conditions consist of a wet season that runs from November to March, and a dry season spanning from April to October. Mean annual rainfall is about 800 mm, with January, February and March being the wettest months, characterized by torrential downpours in the afternoon and sometimes continuous rains for a couple of days. During the rainy season, nights and mornings are fairly comfortable at around 18°C. Afternoon temperatures are around 30°C with relative humidity of 70–80%, making the ecological settings in the study sites ideal for the survival of mosquitoes. The ecological features of the two sites show that they consist mainly of open woodlands, isolated trees and grasses which frequently disappear during the dry season. Selection of the study villages was based on the ecological conditions which provide potential breeding habitats for
both An. gambiae and An. funestus complexes.

Human dwellings are comprised of mud or cement-plastered superstructures with grass-thatched roofs or asbestos/corrugated iron sheets. Domestic animals are sheltered in pens near the homesteads. Most settlements are located near streams or dams or swamps which serve as suitable environments for the breeding of An. funestus mosquitoes. IRS and LLINs remain the pillars for the prevention and control of malaria in both sites, with LLIN ownership close to 100% during the 2013–14 mass distribution campaign (Marevangepo, unpublished data, 2015).

**Mosquito sampling**

Mosquitoes were sampled for one week each month over a period of one year (October 2013 to September 2014) using Centers for Disease Control (CDC) light-traps (John W. Hock Ltd, Gainesville FL., USA), pyrethrum spray collection (PSC) and pit shelters (PS)\(^1\). During each sampling week, light-trap catches were conducted on Tuesday and Thursday nights, while PSC and PS collections were done on Wednesday and Friday mornings. Sampling by light-traps was divided into two cohorts. In one cohort, five indoor and five outdoor light-traps were set up in the evening and left overnight and trapped mosquitoes were collected the following morning. In the other set, two light-traps, one indoor and the other outdoor, were set up in the evening and the trapped mosquitoes were collected hourly till sunrise of the following morning.

For both indoor and outdoor trapping, the light-trap was hung about 1.5 m above the ground close to the feet of an individual sleeping under an insecticide-free (untreated) mosquito net. Two teams each of two people working in six-hour relays aspirated trapped mosquitoes hourly throughout the night. In the two cohorts, light trapping was conducted from 1800 to 0600 hrs for two nights per month at randomly-selected dwellings. The traps installed outdoors were set approximately 10–20 m away from houses. Catches were expressed as the mean proportion of mosquitoes collected per trap per night for overnight and mean proportion of mosquitoes per trap per hour for hourly collections. Indoor and outdoor temperature, relative humidity and rainfall patterns were measured at hourly intervals using a digital thermometer, wet and dry bulb thermometer and rain gauge, respectively.

Centers for Disease Control light-traps were used in this study instead of the standard human landing catch (HLC) method. The light-traps were used as a proxy to approximately measure human biting rates (HBR) of vector mosquitoes since the method has a strong correlation to human landing catches, and three light-traps could collect approximately equal numbers of vectors as two human collectors\(^1\). In addition, it has been shown that where use of vector control interventions is high and vector densities are low, CDC light traps can be used to monitor vector HBR, particularly in case of An. arabiensis, assuming that the mosquitoes that entered a trap during any hour, especially indoors, were those actively seeking a blood meal, and in all probability would bite human hosts in the same hour and area if the light trap was absent\(^1\).

While the golden standard method for assessing HBR is the human landing catches, its extensive use has not been practical in many regions due to the increasing ethical issues and worker safety concerns that this mosquito sampling method increases the risk of exposure of collectors to infectious mosquitoes\(^1\). In addition, the method appears to collect non-standardized data because of variability of the attractiveness and skill of collectors\(^1\).

Pyrethrum spray collection and PS methods were included mainly to determine the human blood index (HBI) and sporozoite rate. Indoor-resting adult mosquitoes were collected by the PSC method\(^1\) in 20 conveniently sampled bedrooms. On each collection day, mosquitoes were collected between 0600 and 1000 hrs from each selected bedroom. Outdoor-resting adult mosquitoes were collected using 20 pit shelters, 10 for each site, conveniently dug in the villages of Burma Valley and Zindi. Pits were separated from each other by a distance of not less than 200 m and located in tree-shaded environments. Each pit shelter was 1.8 m deep, 1.5 m long and 1 m wide, with four small horizontal cavities of 0.3 m deep dug on the walls at a height of 0.6 m from the base. Mosquitoes were aspirated from each PS for two days in each month of the study period.

Female anophelines from all collections were counted, their abdominal status was determined (unfed, partially fed, fully fed or gravid) and were identified using morphological characters\(^4\,16\). Mosquitoes belonging to the An. funestus group and An. gambiae s.l. were placed singly in labeled eppendorf tubes containing silica gel and transported to the National Institute of Health Research (NIHR) laboratory in Harare for further processing.

**PCR species identification**

Polymerase chain reaction (PCR) identification of members of the An. funestus group was determined using DNA extracted from two legs or wings of each morphologically-identified specimen following the method described by Koekemoer et al\(^1\). The PCR assays were to confirm that the assays on blood meal sources and sporo-
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Blood meal source identification and human blood index

All fully blood-fed *An. funestus* mosquitoes collected were assayed using cytochrome b-based multiplex PCR for blood meal source identification\(^\text{18}\). Abdomens of fully fed specimens were separated from the heads and thoraces, ground in 50 μl phosphate-buffered saline and analysed for blood meal sources for human, bovine, dog, goat, and pig primers. HBI was calculated by dividing the number of *An. funestus* mosquitoes with human blood by the total number of *An. funestus* engorged with blood tested. Mixed blood meal sources were each treated as two separate blood meal sources in calculation.

Sporozoite detection in infected mosquitoes and entomological inoculation rate

Heads and thoraces were removed from the abdomens of dried mosquitoes and tested by enzyme-linked immunosorbent assay (ELISA) for the circumsporozoite (CS) protein antigen in the salivary glands using monoclonal antibodies specific for *P. falciparum* following the standard protocol described by Wirtz et al\(^\text{19}\). Tests were conducted to detect only *P. falciparum* because it is the predominant malaria parasite species in Zimbabwe, constituting 95% of all cases\(^\text{1}\). Mosquitoes were pooled and tested in groups of <10 according to collection method and date\(^\text{20–21}\). ELISA positive samples from the initial screening were re-tested to confirm positives and to quantify the amount of CS protein for each sample. Sporozoite rate was obtained by dividing the number of *An. funestus* which contained *P. falciparum* sporozoites with the total number of *An. funestus* tested\(^\text{13}\).

Data analysis

The indoor and outdoor human biting densities and biting times, blood meal sources and differences in sporozoite rates in *Anopheles* mosquitoes collected in the two study sites for the whole sampling period were compared using the one-way ANOVA test at 0.05 level of significance.

Ethical issues

The Director of the Zimbabwe National Malaria Control Programme (NMCP), provincial, district and village authorities as well as household owners were sensitised prior to the study and their permission was sought and obtained. The study protocol was approved by the Ministry of Health and Child Care through the Director of NMCP. All hourly mosquito collectors were provided with untreated mosquito nets during each study night for the entire sampling period. Informed consent and voluntary participation was obtained from hourly mosquito collectors and household owners who participated in the study. The three LLINs were donated to each participating mosquito collector and household during the study.

RESULTS

Anopheline mosquito composition and abundance

Overall, 2268 adult female *Anopheles* mosquitoes were collected by CDC light-traps, PSC and PS methods (Table 1). All the specimens collected by the three methods belonged to two major groups of *Anopheles* mosquitoes—*The An. funestus* group (98.3%) and *An. gambiae* s.l. (1.7%). Of the entire *Anopheles* mosquitoes collected, the majority were caught by CDC light-traps, followed by PSC, and the least by PS. There were no significant differences in the number of *Anopheles* mosquitoes collected at the two study sites (ANOVA: df = 4; F = 2.87; \(p = 0.06\)). All the mosquitoes collected by the CDC light-traps at both sites were unfed. The PSC had higher proportions of fully fed and gravid anopheline mosquitoes than PS method (Table 1).

Indoor and outdoor catches of *Anopheles* mosquitoes

A total of 1096 anopheline mosquitoes were collected by CDC light-traps set indoors and outdoors overnight for the entire one year study period at the two sites (Table 2). The *An. funestus* group constituted the major-

<table>
<thead>
<tr>
<th>Site</th>
<th><em>Anopheles</em> species s.l.</th>
<th>n</th>
<th>CDC</th>
<th>PSC</th>
<th>PS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>UF</td>
<td>FF</td>
<td>G</td>
</tr>
<tr>
<td>Burma Valley</td>
<td><em>An. funestus</em> s.l.</td>
<td>1139</td>
<td>69</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>An. gambiae</em> s.l.</td>
<td>21</td>
<td>71.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Zindi</td>
<td><em>An. funestus</em> s.l.</td>
<td>1090</td>
<td>63.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>An. gambiae</em> s.l.</td>
<td>18</td>
<td>61</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

CDC—Centres for Disease Control and Prevention; PSC—Pyrethrum spray collection; n—Number; UF—Unfed; FF—Fully fed; G—Gravid.
A percentage of the total anopheline mosquitoes collected. Of these, 68.8% were sampled indoors and the remaining were caught outdoors. The indoor and outdoor biting densities for the *An. funestus* group and *An. gambiae* s.l. were significantly different (ANOVA: df = 1; F = 12.93; p = 0.01). Comparison of flight activities for *An. funestus* between sites revealed no significant differences in mosquito numbers both indoors (ANOVA: df = 1; F = 0.02; p = 0.89) and outdoors (ANOVA: df = 1; F = 0.01; p = 0.90).

### Seasonal occurrence of anopheline mosquitoes

The indoor and outdoor CDC light catches of the *An. funestus* group and *An. gambiae* s.l. varied according to the season of the year (Table 3), with approximately light-trap collection densities of 3.3 and 2.0 mosquitoes per trap per night during the wet season (November to March) for *An. funestus* mosquitoes in Burma Valley and Zindi, respectively. Most of the *An. funestus* group and *An. gambiae* s.l. mosquitoes were collected during the wet season at both sites. *Anopheles funestus* indoor catches during the dry season (April to October) was lower than those in wet season. In general, the occurrence of *An. funestus* populations persisted from late wet to early dry season and were completely absent in the mid-dry season which coincided with winter. Comparison of seasonal biting activity for *An. funestus* populations and *An. gambiae* s.l. revealed significant differences in mosquito densities sampled indoors and outdoors between seasons (wet and dry) (ANOVA: df = 4; F = 8.65; p = 0.0).

### Biting rhythm of *Anopheles funestus* group in the villages of Burma Valley and Zindi

In both study sites, the majority of adult female *An. funestus* collected indoors and outdoors exhibited flight activity almost throughout the night (Fig. 1). Indoor and outdoor mosquito flight activity commenced at 2000 hrs, but steadily increased up to 2300 hrs, and decreased either gradually or sharply thereafter to the point of almost zero flight around 0100 hrs. However, flight activity peaks varied slightly at collection sites, with two peaks: The first peak during the first six hours of the night and the second during the last six hours. From 0200 hrs, the indoor flight activity rhythm increased steadily with peak observed between 0200 and 0300 hrs in Burma Valley and 0200 to 0400 hrs in Zindi. The major flight activity periodicity occurred during the second half of the night. The observed indoor flight activity of the *An. funestus* group exceeded the outdoor flight activity at both sites and there were significant differences between the indoor and outdoor flight activity (ANOVA: df = 3; F = 8.25; p < 0.01). The outdoor flight activities of the *An. funestus* group were apparently similar in both areas, except for a sharp fall after 0300 hrs in Burma Valley.

### Table 2. Percentage variation of indoor and outdoor biting behaviour of *Anopheles* mosquitoes

<table>
<thead>
<tr>
<th>Site</th>
<th>n</th>
<th><em>An. funestus</em> group</th>
<th>n</th>
<th><em>An. gambiae</em> complex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Indoor</td>
<td>Outdoor</td>
<td>Indoor</td>
<td>Outdoor</td>
</tr>
<tr>
<td>Burma Valley</td>
<td>588</td>
<td>68.5</td>
<td>31.5</td>
<td>13</td>
</tr>
<tr>
<td>Zindi</td>
<td>488</td>
<td>69.1</td>
<td>30.9</td>
<td>7</td>
</tr>
</tbody>
</table>

n—Number.

### Table 3. Seasonal biting densities of indoor and outdoor anopheline mosquitoes in Burma Valley and Zindi sites

<table>
<thead>
<tr>
<th>Site</th>
<th>Species</th>
<th>Period</th>
<th>Indoor No. of traps</th>
<th>Density (Range)</th>
<th>SE</th>
<th>Outdoor No. of traps</th>
<th>Density (Range)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burma Valley</td>
<td><em>An. funestus</em></td>
<td>Wet season (Nov–Mar)</td>
<td>25</td>
<td>4.82 (3.31–6.63)</td>
<td>0.65</td>
<td>25</td>
<td>1.8 (1.12–2.43)</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dry season (Apr–Oct)</td>
<td>24</td>
<td>2.43 (0–5.92)</td>
<td>0.88</td>
<td>23</td>
<td>1.36 (0–4.71)</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td><em>An. gambiae</em> s.l.</td>
<td>Wet season (Nov–Mar)</td>
<td>25</td>
<td>0.16 (0–0.53)</td>
<td>0.12</td>
<td>25</td>
<td>0.1 (0–0.20)</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dry season (Apr–Oct)</td>
<td>24</td>
<td>0.09 (0–0.50)</td>
<td>0.07</td>
<td>23</td>
<td>0.03 (0–0.23)</td>
<td>0.03</td>
</tr>
<tr>
<td>Zindi</td>
<td><em>An. funestus</em></td>
<td>Wet season (Nov–Mar)</td>
<td>23</td>
<td>3.84 (2.93–5.33)</td>
<td>0.45</td>
<td>23</td>
<td>1.86 (1.01–2.73)</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dry season (Apr–Oct)</td>
<td>24</td>
<td>1.10 (0–1.82)</td>
<td>0.82</td>
<td>23</td>
<td>0.38 (0–1.52)</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td><em>An. gambiae</em> s.l.</td>
<td>Wet season (Nov–Mar)</td>
<td>23</td>
<td>0.52 (0–1)</td>
<td>0.23</td>
<td>22</td>
<td>0.10 (0–0.31)</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dry season (Apr–Oct)</td>
<td>24</td>
<td>0.46 (0–1.91)</td>
<td>0.27</td>
<td>23</td>
<td>0.08 (0–0.32)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

SE—Standard error.
PCR analysis of the Anopheles funestus sibling species

A total of 726 An. funestus group mosquitoes which were either fully blood-fed or gravid were PCR-assayed for identification to sibling species. The analysis revealed that 91.4% were An. funestus s.s., 4.9% An. leesoni, while 3.7% could not be identified despite the successful amplification of positive control. However, fully blood-fed and gravid An. gambiae s.l. specimens were not PCR-tested to sibling species due to non-availability of budget to procure primers to differentiate members of the An. gambiae complex.

Identification of blood meal sources of Anopheles funestus

The PCR diagnostic identified 272 blood meals of humans and domestic animals from engorged An. funestus collected from the two study sites (Fig. 2). The results indicated that the majority of An. funestus fed on human blood source (anthropophily), resulting in HBI of about 0.64 (n = 175) for both indoor and outdoor resting mosquitoes collected at the two sites (Table 4). The remaining proportion of blood meals was received from domestic animals (zoophily). Among the animals, An. funestus showed fairly high preference for bovine blood, with dog blood least preferred. Only one specimen showed mixed bovine and goat blood sources.

Detection of circumsporozoite antigen

Table 5 shows the total number of An. funestus specimens tested for P. falciparum circumsporozoite antigen and infection rates using ELISA. Only about 2% (n = 8) of the specimens tested positive for P. falciparum sporozoites among the mosquitoes collected from the two study sites. Partitioning the infection rates by site indicated a close to 2% sporozoite rate in An. funestus for Burma Valley and about 1% for Zindi.

DISCUSSION

Work by Lines et al. in Tanzania and Fornadel et al. in Zambia demonstrated that CDC light-trap is comparable to human landing catches, and therefore, considered a proxy tool for sampling malaria vector mosquitoes that would otherwise feed on humans. While the light-trap has been shown to underestimate the actual mosquito biting risk, CDC light-traps are important as they can be used to monitor HBRs in malaria vector mosquitoes in areas where use of vector control interventions is high, with low vector densities. Mosquitoes caught by CDC light-traps in the present work is comparable to those reported in similar studies where each trap was set next to a human and that the majority of the mosquitoes collected were unfed, suggesting that the mosquitoes were caught in the act of host-seeking.

Investigating host-seeking behaviour, host preferences and presence of sporozoites in Anopheles mosquitoes are necessary to understand their probability as vectors of malaria. A study by Sharp demonstrated that the biting behaviour of Anopheles mosquitoes can be markedly disrupted by changes in environmental factors during the night, especially rain and wind. Wind is known to have a direct effect on mosquito flight. However, no major adverse weather conditions were encountered during the entire period of the study and it was possible to collect mosquitoes at different venues and time, which indicated important entomological information that could be utilized to implement appropriate vector control interventions.
In Burma Valley and Zindi, *An. funestus* was found to be the major anopheline and the species demonstrated predominantly indoor biting patterns. An estimate of the degree of endophagy and exophagy can be obtained when the relative proportions of the mosquitoes attempting to bite indoors and outdoors are compared\textsuperscript{27}. The CDC light catches in this study demonstrated that mosquitoes were more abundantly indoors (68\%) than outdoors (32\%), suggesting that the indoor nocturnal host-seeking tendencies of *An. funestus* and *An. gambiae* s.l. could be interrupted by the intra-domiciliary use of LLINs by the majority of residents of Burma Valley and Zindi areas. However, the relevance of outdoor host-seeking behaviour of mosquitoes to vector control might depend largely on the coincidence between outdoor biting intensity and human outdoor activity\textsuperscript{28}.

Comparative account of indoor and outdoor biting profiles for malaria vector mosquitoes in Zimbabwe are lacking from published literature. However, the results of the present study are consistent with the previous studies in Uganda in which most *An. funestus* populations and *An. gambiae* s.l. fed indoors\textsuperscript{28}. The finding of this work is not in agreement with other studies which reported outdoor host-seeking profiles in *An. arabiensis* from Nigeria\textsuperscript{29}, and *An. gambiae* s.s. from Bioko Island, Equatorial Guinea\textsuperscript{30}. In other example *An. neivai* in Colombian Pacific\textsuperscript{31}, fed outdoors following exposure to insecticide pressure. Although, this study showed that majority of vector mosquitoes were collected in indoor CDC light-trap catches, the densities appear to be strongly dependent upon seasons (wet or dry).

In the present work, seasonal mosquito biting profiles were described and categorised into wet and dry seasons. The densities of *An. funestus* populations and *An. gambiae* s.l. were higher during the wet season (*i.e.* February/March in Zimbabwe) than dry season when vector density is generally at its peak following abundance of breeding sites, favourable temperatures and relative humidity\textsuperscript{10}.

Little is known about the seasonal biting behaviour of malaria vectors in Zimbabwe for comparative purposes. However, results of this study are comparable to those reported in Nigeria for *An. gambiae* but with *An. funestus* having high dry seasonal biting tendencies\textsuperscript{29}. The results of the current study suggest that while it is important to conduct mass and continuous net distribution campaigns, as well as net hang-up campaigns all year round, it is critical to intensify net hang-up campaigns in wet season.

When the indoor and outdoor components of hourly trap catches of *An. funestus* populations were examined, it was noted that the rhythm of flight activity fluctuated in a similar fashion throughout the night in both sites. Knowledge on the biting times of anopheline mosquitoes is crucial in ascertaining whether peak biting period coincides with that part of the night after the inhabitants retired to bed. An important finding in this context was the general nocturnal mosquito flight cycles.

The first biting activity peak which occurred prior to midnight (2200–2300 hrs) suggests the possibility of continued malaria transmission despite net ownership and use, as this was a period when probably a fairly small proportion of the rural population might still be out of bed. Further, the second peak was observed towards dawn (0300–0400 hrs), a period which might put some people at risk of mosquito bites as they might be out of bed for early morning household chores. This suggests that the use of mosquito repellents would be effective to complement LLINs during the double peaks when some people would not be in bed under LLINs.

Although, Moiroux et al\textsuperscript{32} also observed two peaks of biting activity of *An. funestus*, the times of the first and second peaks which were recorded between 12-00 hrs, and 0300 and 0400 hrs, respectively, differed from the peak times of this study. In contrast, in Masakadza area, Zimbabwe, Dandalo\textsuperscript{11} reported that biting of *An. gambiae* complex mosquitoes commenced at 1900 hrs and ceased at 0500 hrs with biting peaks at 2200 hrs. In Kenya the biting of *An. gambiae* s.l. gradually increased throughout the night with a peak three hours before dawn\textsuperscript{33}. However, the explanations for the two biting peaks and the sharp fall in biting behaviour at 0100 hrs were unclear and could not be established from the present work.

The risk of transmission of mosquito-borne diseases to human populations depends greatly on the degree of biting on humans by vector mosquitoes, which in turn would be influenced by abundance, distribution, and host blood source preferences. In relation to the host blood meal sources, the degree of risk would depend largely on the anthropophilic or zoophilic profile of the vector mosquito. More than 64\% of the blood meals identified from *An. funestus* collected from Burma Valley and Zindi were obtained from human host, suggesting that this population of *An. funestus* is highly anthropophilic, and this tendency to feed on human blood increases vectorial capacity\textsuperscript{34}. The high HBI in the present study might be attributed to the attraction of the species to human habitats where no domestic animals are kept. Human blood index in *An. funestus* species observed in this study is consistent with maintaining high levels of malaria transmission in almost total absence of other vector species and is an important factor in the epidemiology of the disease as well as in estimating human-vector contact for determining malaria risk.
transmission intensity and planning for its control. However, large proportion of mixed human and animal blood meals widely reported in other studies on An. funestus mosquitoes35-37, and An. arabiensis34,38 were not observed in the present work. However, this sharp contrast could not be clearly understood.

The sporozoite rate of about 2% in An. funestus in Burma Valley and Zindi specimens was low. Annual P. falciparum infectious bites lower than 10% sporozoite rate indicate its unstable transmission intensity39 and risk of epidemics40. Basing on the studies by Okello et al39 in Uganda and Lindsay and Martens40 in African highlands, the results from the current study suggest that malaria transmission in Burma Valley and Zindi might be unstable with possibility of spontaneous epidemics which calls for vigilant surveillance to avert unforeseeable disasters.

CONCLUSION

In Burma Valley and Zindi areas, malaria control strategies have greatly targeted intra-domiciliary vector mosquitoes largely through the provision of LLINs with net ownership of about 100% apiece. This tool has been proved effective against epidemiologically important anopheline vectors with prominently indoor biting behaviour. However, where human biting occurs outdoors and/or before midnight and/or towards dawn when people are not protected by LLINs, indoor-based mosquito net intervention might not be sufficient to reduce malaria incidence to a point where it is no longer a public health problem. An important finding in this context was that generally, the nocturnal mosquito flight cycles commenced at 2000 hrs, with double peaks between 2200 and 2300 hrs during the first six hours of the night, and between 0200 and 0400 hrs for the second six hours. By 0600 hrs, flight activities almost completely ceased. As such, it is clear from the results of this study that consistent use of nets every night all year round, use of personal protective clothing and repellents during peak mosquito densities might suppress malaria transmission. More so, the biting patterns of the An. funestus populations warrant further study.

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